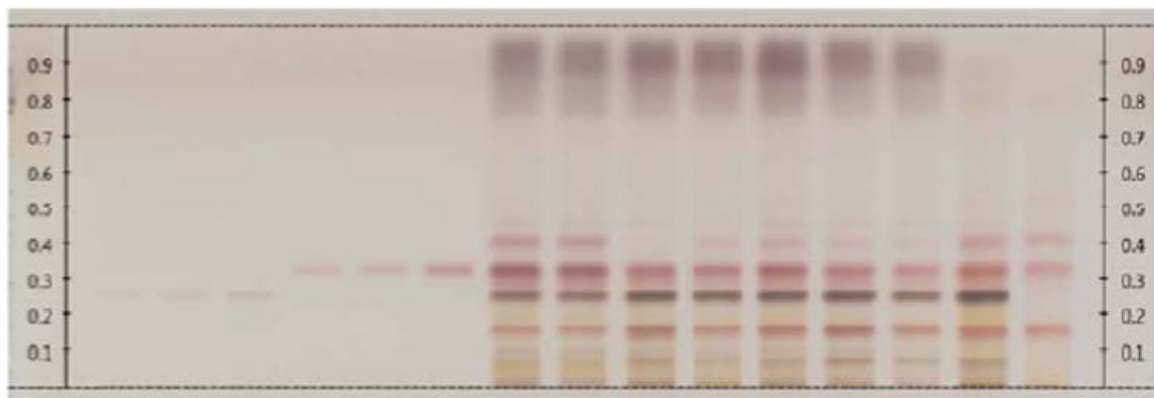


## ***Vitex negundo* Leaf – Identification**

### **Thin-Layer Chromatography**



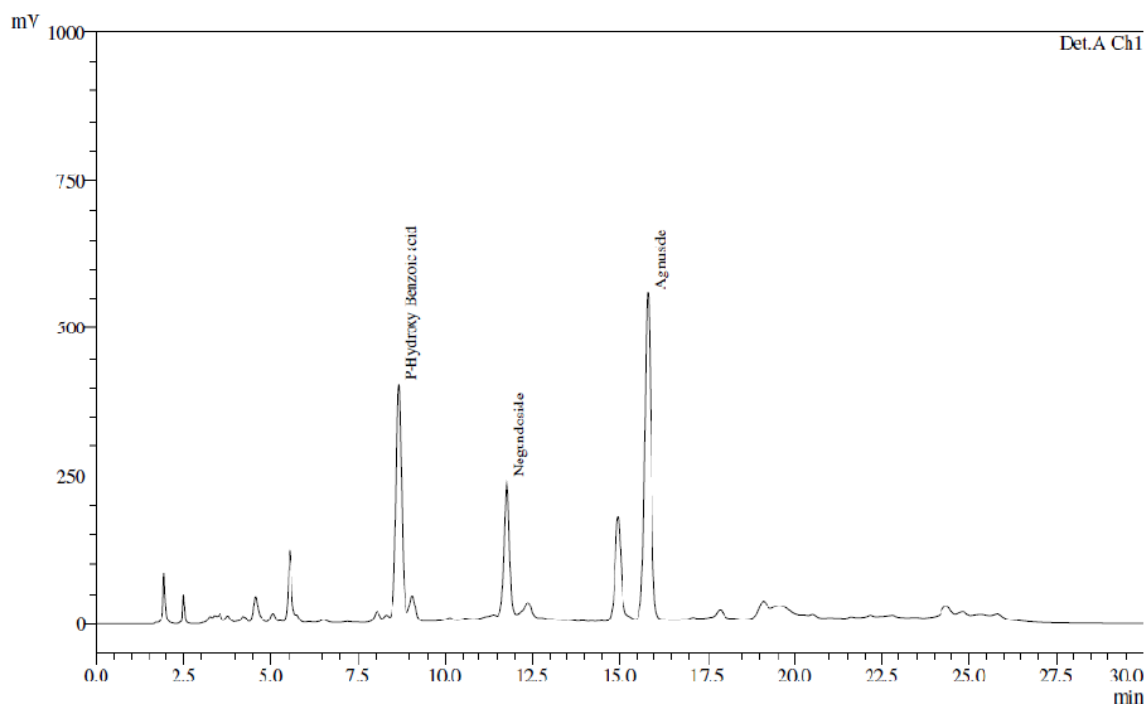
**Typical HPTLC Chromatogram**

***These chromatograms are supplied for information only***

**Track assignment:** 1-3) Agnuside (3 mg/mL); 4-6) Negundoside (3 mg/mL); 7-13) *Vitex negundo* Leaf, commercial samples; 14- 15) *Vitex negundo* Leaf Dry Extracts, commercial samples (30 mg/mL).

|                                   |                                                                                                                                                             |
|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Sample solutions:</b>          | according to the monograph                                                                                                                                  |
| <b>Standard solutions:</b>        | in methanol                                                                                                                                                 |
| <b>Plate:</b>                     | HPTLC, Silica gel 60 F <sub>254</sub> , 5 µm                                                                                                                |
| <b>Saturation time:</b>           | 20 minutes                                                                                                                                                  |
| <b>Application volume:</b>        | 5 µL, as 8-mm bands                                                                                                                                         |
| <b>Relative Humidity:</b>         | about 33%                                                                                                                                                   |
| <b>Developing solvent system:</b> | ethyl acetate, glacial acetic acid, and water (80:10:5)                                                                                                     |
| <b>Developing distance:</b>       | 7 cm                                                                                                                                                        |
| <b>Derivatization reagent:</b>    | anisaldehyde-sulfuric acid reagent (a mixture of 170 mL of ice cold methanol with 20 mL of glacial acetic acid, 10 mL sulfuric acid, and 1 mL anisaldehyde) |
| <b>Detection:</b>                 | derivatize, heat at 100°C for 3 min, and examine under visible light.                                                                                       |

## HPLC (Negundoside)



**Representative chromatogram of Content of Iridoid Glycosides in *Vitex negundo* Leaf**

*This chromatogram is supplied for information only*

|                              |                                                                                                                                     |
|------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|
| <b>Solution preparation:</b> | according to the monograph                                                                                                          |
| <b>Mode:</b>                 | HPLC                                                                                                                                |
| <b>Detector:</b>             | UV, 254 nm                                                                                                                          |
| <b>Column:</b>               | 4.6-mm × 25-cm; 5-μm packing L1 (Similar to Lichrospher 100 RP 18)                                                                  |
| <b>Column temperature:</b>   | 25°±1                                                                                                                               |
| <b>Flow rate:</b>            | 1.5 mL/min                                                                                                                          |
| <b>Injection volume:</b>     | 20 μL                                                                                                                               |
| <b>Solution A:</b>           | dissolve 0.14 g of potassium phosphate monobasic in 900 mL of water,<br>add 0.5 mL of o-phosphoric acid, complete to 1 L with water |
| <b>Solution B:</b>           | acetonitrile                                                                                                                        |
| <b>Mobile phase:</b>         | see <i>Table 1</i>                                                                                                                  |

**Table 1**

| <b>Time<br/>(min)</b> | <b>Solution A<br/>(%)</b> | <b>Solution B<br/>(%)</b> |
|-----------------------|---------------------------|---------------------------|
| 0.01                  | 90                        | 10                        |
| 10                    | 85                        | 15                        |
| 20                    | 80                        | 20                        |
| 23                    | 80                        | 20                        |
| 25                    | 85                        | 15                        |
| 30                    | 90                        | 10                        |
| 35                    | 90                        | 10                        |